CARD8 activation by HIV-1 PR triggers GSDMD-dependent and -independent cell death

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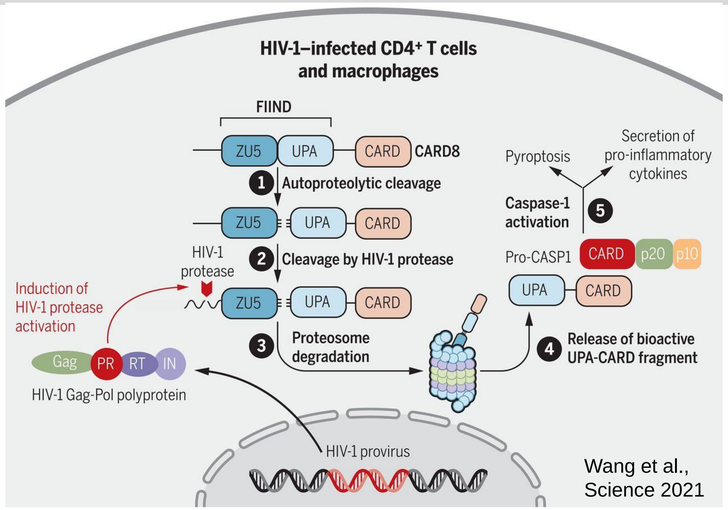
Introduction

Recently, we reported that induction of HIV-1 protease (PR) activation leads to CARD8 inflammasome-mediated pyroptosis of infected macrophages and CD4+ T cells. In macrophages, it is clear that caspase-1 (CASP1)-dependent inflammasome activation results in the cleavage and activation of gasdermin D (GSDMD), which then triggers a lytic inflammatory cell death called pyroptosis. It remains unclear whether CARD8 inflammasome induced killing of HIV-1-infected CD4+ T cells is GSDMD-dependent or other cell killing mechanisms are involved.



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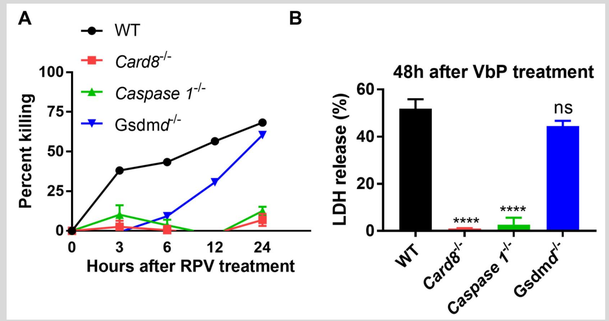
Methods

We treated HIV-1-infected THP-1 cells and primary CD4+ T cells with non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as rilpivirine (RPV) and efavirenz (EFV) to induce HIV PR-mediated CARD8 inflammasome activation and we also treated uninfected THP-1 cells and primary CD4+ T cells with DPP9 inhibitor Val-boroPro (VbP) to activate the CARD8 inflammasome and investigated the mechanisms of cell death in control and GSDMD-KO cells.

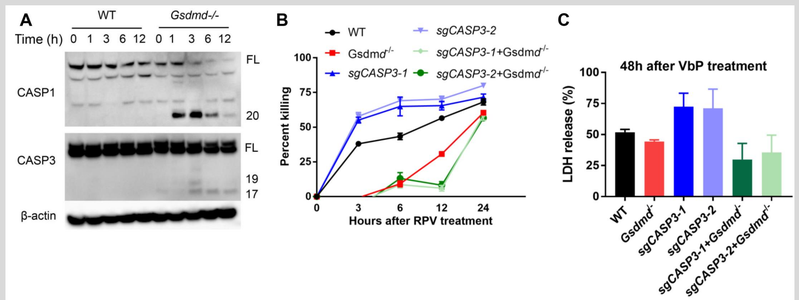
Results

Fig. 1 GSDMD deficiency only delays cell death after CARD8 inflammasome activation



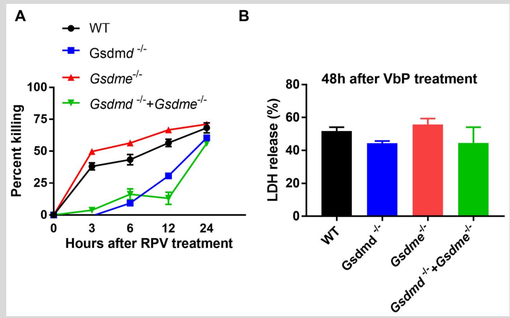
(A) WT, *Card8*$^{-/-}$, *Caspase 1*$^{-/-}$ and *Gsdmd*$^{-/-}$ THP-1 cells were infected with HIV-1-GFP reporter virus for 3 days and then treated with RPV for 3 to 24 hours. GFP+ cells were detected by flow cytometry. (B) LDH release from WT, *Card8*$^{-/-}$, *Caspase 1*$^{-/-}$ and *Gsdmd*$^{-/-}$ THP-1 after VbP treatment.

Fig. 2 GSDMD-independent cell death is only partially driven by Caspase 3 activation



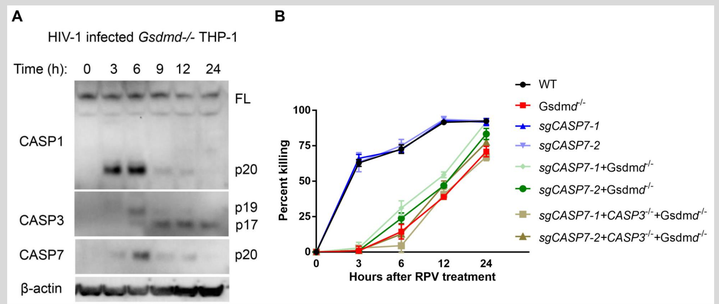
(A) Immunoblot showing Caspase-1/3 processing from HIV-1 infected *Gsdmd*$^{-/-}$ THP-1 after RPV treatment. (B) Percent killing from WT, *Caspase 3*$^{-/-}$ and *Gsdmd*$^{-/-}$*/Caspase 3*$^{-/-}$ HIV-1 infected THP-1 after RPV treatment. (C) LDH release from WT, *Caspase 3*$^{-/-}$ and *Gsdmd*$^{-/-}$*/Caspase 3*$^{-/-}$ THP-1 after VbP treatment.

Fig. 3 GSDME does not contribute to cell death after CARD8 inflammasome activation



(A) WT, *Gsdmd*$^{-/-}$,*Gsdme*$^{-/-}$ and *Gsdmd*$^{-/-}$*/ Gsdme*$^{-/-}$ THP-1 cells were infected with HIV-1-GFP reporter virus for 3 days and then treated with RPV for 3 to 24 hours. GFP+ cells were detected by flow cytometry. (B) LDH release from WT, *Gsdmd*$^{-/-}$,*Gsdme*$^{-/-}$ and *Gsdmd*$^{-/-}$*/ Gsdme*$^{-/-}$ THP-1 after VbP treatment.

Fig. 4 Caspase 3 and Caspase 7 ablation do not completely abolish GSDMD-independent cell death



(A) Immunoblot showing Caspase-1/3/7 processing from HIV-1 infected *Gsdmd*$^{-/-}$ THP-1 after RPV treatment. (B) WT, *Gsdmd*$^{-/-}$, *Casp7*$^{-/-}$ and *Casp3/7*$^{-/-}$*/Gsdmd*$^{-/-}$THP-1 cells were infected with HIV-1-GFP reporter virus for 3 days and then treated with RPV for 3 to 24 hours.

Conclusion

This work reveals that HIV-1-infected cells can undergo CASP1-induced lytic cell death after CARD8 inflammasome activation in GSDMD-dependent and -independent manners. This study broadens our understanding of CARD8 inflammasome-induced cell death and has implications for the clearance of HIV-1 reservoir cells in patients.